Aus dem Lehrstuhl für Klinische Biochemie
der Universität Düsseldorf
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Direktor: Prof. Dr. med. H. Reinauer

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Inaugural-Dissertation
zur Erlangung der Doktorwürde
der Medizinischen Fakultät der Universität Düsseldorf

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Influence of streptozotocin-diabetes on myocardial lipids and prostaglandin release by the rat heart.

Key words: rat heart, lipid metabolism, prostacyclin, PGE_2, fatty acid composition of myocardial lipids, Streptozotocin-diabetes

Abbreviations: PG prostaglandin, SZ streptozotocin

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Summary

To elucidate the influence of a diabetic state on the formation of prostacyclin, hearts from acutely (100 mg/kg streptozotocin, 3 days) and chronically diabetic rats (60 mg/kg streptozotocin, 30 days) were perfused by the Langendorff-technique. The release of prostacyclin, 6-keto-$PGF_{1\alpha}$ and PGE_2 was determined in the cardiac effluate by bioassay and RIA. The basal release of 6-keto-$PGF_{1\alpha}$ was reduced by the diabetic state for about 40-50%, the capacity for formation of prostacyclin and PGE_2 (measured after application of arachidonic acid) was, however, increased 2-3-fold in acutely and chronically diabetic rat hearts. Determination of myocardial lipids showed an elevation of the free fatty acid fraction in acutely and chronically diabetic hearts. Triglycerides were, however, only increased in the acutely-diabetic, ketotic state. The amount of phospholipids was not changed by diabetes. The fatty acid spectrum of the various lipid fractions showed significant alterations mainly in the free fatty acid fraction and in triglycerides, but not in phospholipids. From these data we conclude that the reported changes in myocardial PG-formation by diabetes cannot be explained by alterations in the enzymatic activities of cyclooxygenase and prostacyclin-synthase, but reflect changes in the availability of arachidonic acid. We think that the reduced availability of free arachidonic acid does not result from an absolute or relative loss of arachidonic acid in myocardial lipids, but that the activity and/or regulation of phospholipases delivering arachidonic acid for PG-synthesis is modified by diabetes.

INTRODUCTION

A higher incidence of cardiovascular diseases has been described in diabetic patients (1,2,3). Changes in the formation of prostacyclin, a most potent vasodilator and inhibitor of platelet aggregation and deposition (4) and in the synthesis of thromboxane A_2 by the platelets favouring their aggregation and deposition (5) were suggested to contribute to the development of vascular disturbancies and the increased platelet aggregability demonstrated in diabetic patients (6). Reports regarding prostaglandin-biosynthesis are, however, controvers (7-17). We observed an increased prostacyclin and PGE_2 synthesis by acutely diabetic hearts after application of arachidonic acid (7). Elevated levels of PGE_2 were found in the cardiac effluate of diabetic hearts (7a) and in serum of diabetic children (15). On the other hand, Harrison and others (10-12,16,17) reported an impaired release of prostacyclin by isolated large vessels of diabetic patients and rats.

Therefore, this study has been undertaken (a) to prove whether the basal release of prostacyclin by heart is impaired in diabetes. (b) To test whether the capacity of PG biosynthesis is changed by diabetes, release and, thereby, biosynthesis of PGs was measured applying increasing dosages of arachidonic acid. PG release at high concentration of arachidonic acid should reflect the capacity for PG biosynthesis in heart. To exclude, furthermore, that the increased release of prostacyclin described by us (7) resulted from acutely diabetic decompensation (3 days after injection of streptozotocin (SZ), the prostacyclin release was studied additionally in "chronically" (30 days after application of SZ) diabetic rat hearts.

(c) Furthermore, since changes in lipid metabolism by diabetes (18-20) and in the fatty acid composition might influence the availability of precursor fatty acids for PG-synthesis, we studied the myocardial content of phospholipids, triglycerides and non-esterified fatty acids as well as the individual fatty acid composition of these lipid fractions.

METHODS:

Materials:
Enzymes for the analysis of substrates, coenzymes and streptozotocin were purchased from Boehringer (Mannheim), heptadecanoic, eicosadienoic acid and triheptadecanoin from Applied Science Europe (Heidelberg). Arachidonic acid were supplied by Nu-Chek Inc.(Elysian, Minnesota, >99% pure), prostacyclin and PGE_2 by the Upjohn Comp. (Kalamazoo, Michigan), indomethacin by Merck, Sharp & Dohme (München). The preparation of arachidonic acid, prostacyclin and PGE_2 as well as of the blocking agents has been described elsewhere in detail (7,19,20) 6-keto-$PGF_{1\alpha}$ and the antibody for the radioimmunological determination were delivered by NEN (Boston, USA). All other chemicals were of analytical grade.

Animals:
Male Wistar rats (strain II, 260-330 g body weight) were fed a standard diet (Sniff, Soest) and received water ad libitum. Diabetes was induced by application of SZ (Boehringer, Mannheim) dissolved in 0.1 M citrate buffer, pH 4.5, immediately before use. Blood glucose was determined by the glucose oxidase method, glucosuria and ketonuria were routinely checked in the urine by "Glucetur" sticks (Boehringer).
Rats were studied in two different diabetic states:

(a) 3 days after application of SZ (100 mg/kg intraperitoneally) a severe metabolic disorder was observed according to the following criteria: hyperglycemia, glucosuria, reduced plasma insulin concentrations, ketonemia and ketonuria associated with a rapid loss in body weight and hypertriglyceridemia ("acutely diabetic", 21).
(b) A lower dose of streptozotocin (60 mg/kg body weight) induced 30-35 days after application a more chronically diabetic state characterized by hyperglycemia, glucosuria and reduced in-

sulin plasma concentrations, but no ketonemia and ketonuria ("Chronically diabetic", 21).
In both cases, treatment with insulin (4 I.U. per day) led to a complete normalization of blood glucose levels and prevented glucosuria, ketonemia and the loss in body weight.

Preparation of the hearts and isolation of myocardial lipids: Rats were briefly anaesthetized with ether and the hearts were quickly excised after thoracotomy and, immediately, freeze-clamped with aluminium blocks precooled in liquid nitrogen. Atrial and non-ventricular tissue was removed from the heart by a dental drill.
The frozen heart ventricles were pulverized in a precooled mortar, homogenized in $CHCl_3/CH_3OH$ (2:1,v/v) by a Ultra-Turrax (Jahnke & Kankel, Staufen), and extracted overnight according to the procedure of Folch et al.(22) after addition of a defined amount of heptadecanoate and triheptadecanoin as internal standards. After centrifugation the supernatant was filtered through glas wool and brought to dryness under a stream of nitrogen. If necessary, the dried lipids were stored under nitrogen at $4^{\circ}C$. The dried lipids were dissolved in n-hexane and chromatographed on silicagel(DC Kieselgel 60, Merck) using hexane, ether, acetic acid (80:20:1,v/v/v) as solvent system. After development the phospholipid, triglyceride and free fatty acid fractions were identified by external standards. The appropriate zones were scrapped off and eluted with $CHCl_3/CH_3OH$. After addition of heptadecanoate to the isolated phospholipid fraction the methylation of the fatty acids was carried out by the method of Morrison and Smith (23) adding boron trifluoride to the dried lipid fractions (phospholipids: 3ml, 14%, free fatty acids: 3 ml, 20%, triglycerides: 1ml, 14%, additionally 0.8ml n-hexane and 2.2ml CH_3OH). The tubes were securely sealed and then heated to $110^{\circ}C$: 5 min reaction time for free fatty acids, 20 min. for phosphoplipids and 30 min. for triglycerides. After cooling and concentration the esterified fatty acids were extracted by 10 ml

n-hexane. The hexane phases were evaporated under a stream of nitrogen. Immediately before use, the dried fatty acis esters were dissolved in n-heptane and determined by gaschromatography (Siemens L350, capillary column, 50m, WS11, WGA,Griesheim-Hiss). Fatty acids were identified with known standards.Peak areas were computed by integrator (Spectraphysic, Darmstadt) and the fatty acids were calculated according to the internal standard (24). In some cases, the gaschromatographic peaks were additionally identified by mass spectrometry (Varian, MAT, Bremen).

Glycerides were measured after alkaline saponification with the glycerolkinase-method (25).

Prostaglandins

Spontaneously beating rat hearts were perfused with Krebs-Henseleit-bicarbonate puffer saturated with 5% CO_2 in oxygen and prostacyclin and PGE_2 release was determined in the cardiac effluent by the differential bioassay using a chick rectum (PGE_2) and bovine coronary artery (prostacyclin) superfused in series with the cardiac effluate and a mixture of blocking agents as previously described (7,26,27).
6-keto-PGF_1 was measured in aliquots of the cardiac effluate by radioimmunoassay. Briefly, appropriately diluted samples were incubated overnight at 4°C with antiserum to 6-keto-$PGF_{1\alpha}$. Free tracer was separated from the antibody bound tracer by precipitation using the polyethylen-glycol method. After washing the precipitates were dissolved in 2N KOH (0.5ml) and the radioactivity determined after addition of acetic acid (0.5 ml) in 10 ml liquid scintillation cocktail (Quickszint, Zinser, Frankfurt) by a ß-counter (Kontron, Münchenstein, Switzerland). All samples were assayed in duplicate in a single assay. Cross reaction was determined to PGE_2 as 3%, intraassay variation was less than 5%.

Calculations and statistics:

The data are given as mean + standard error of mean. Statistical significance was tested by Student's t-test. $p < 0.05$ was assumed as statistically significant.

RESULTS:

Prostaglandin release:

The release of 6-keto-$PGF_{1\alpha}$ determined by radioimmunoassay in the cardiac effluate showed a tendency to be reduced to about 40-50% in the diabetic states, independently of the duration of SZ application (Tab.1). Similar changes were observed when the release of prostacyclin by aortae from control and diabetic rats was measured (not shown).
To decide whether this reduced basal release of PG-like material by diabetic hearts might indicate a diminished enzymatic activity of the enzymes involved (cyclooxygenase, prostacyclin-synthase) or a decrease in the amount of precursor fatty acids for PG-biosynthesis, hearts of control and diabetic rats were infused with increasing dosages of arachidonic acid. Furthermore, to exclude that the observed changes in PG-release might result from an accelerated degradation of prostacyclin to 6-keto-$PGF_{1\alpha}$ or from an alteration of the ratio of releases PGs, a bioassay method was used in these studies indicating directly the amount of prostacyclin and not the degradation product.
Arachidonic acid administration resulted in an immediate decrease in coronary perfusion pressure and the release of PG-like substances, relaxing bovine coronary arteries, but contracting chick rectum and rat stomach. Treating the hearts with indomethacin (2 μg/ml) completely prevented this arachidonic acid induced release of PG-like substances. The dose response curves for the release of prostacyclin- and PGE_2-like activities by

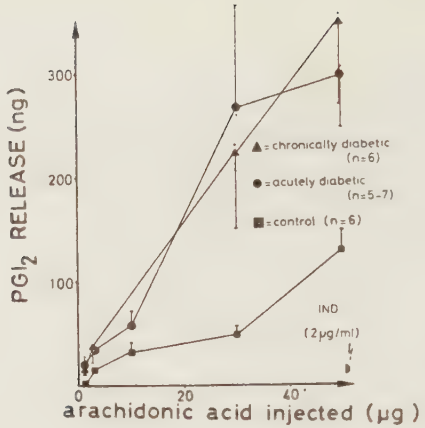

Fig.1: Release of prostacyclin from control, acutely and chronically diabetic rat hearts.

The prostacyclin was measured by the relaxation of bovine coronary artery strip. The hearts were perfused as described in the Method section, and arachidonic acid was injected into the aortic inflow tract after an equlibration period of 20-30 min. means ± SEM of 3-7 experiments are indicated.

■ control, ● acutely diabetic, ▲ chronically diabetic, ◐ pretreatment of the hearts with indomethacin (2 ug/ml).

\+ $p < 0.05$

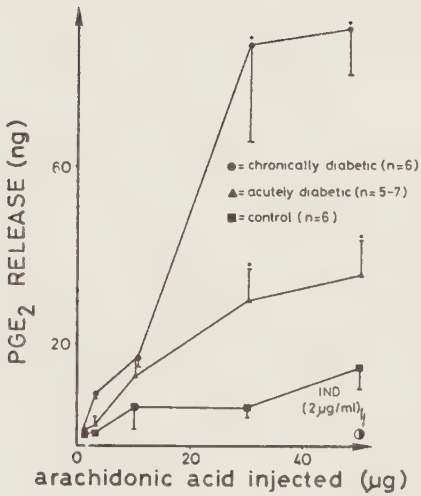

Fig.2: Release of PGE_2 from control, acutely and chronically diabetic rat hearts.
The PGE_2 was measured by contraction of chick rectum. Further details are given in the Method section and in the legend to fig.1. ■ control, ▲ acutely diabetic, ● chronically diabetic, ◐ pretreatment of the hearts with indomethacin (2 µg/ml).
+ $p < 0.05$

hearts of acutely, chronically diabetic and control rats are shown in Fig.1 and 2. Prostacyclin was the major prostaglandin released by the heart after application of arachidonic acid (30). Moreover, the prostacyclin release was increased 2-3 fold from acutely as well as from chronically diabetic hearts as compared to controls. However, the amount of prostacyclin released from acutely diabetic hearts was not significantly different from that produced by chronically diabetic rat hearts (Fig.1). Regarding the PGE_2-like activities, acutely and chronically diabetic rat hearts released more PGE_2-like activities than controls, but in contrast to prostacyclin release, a further increase in the production of PGE_2-like material was observed in hearts of chronically diabetic hearts (Fig.2). Consequently, the ratio prostacyclin/PGE_2 was changed depending on the dose of arachidonic acid applied in hearts of chronically diabetic rats, but not in those of acutely diabetic and control rats (Tab.2). A contraction of rabbit aorta indicating release of PG-endoperoxides or thromboxane A_2 was observed neither in hearts of diabetic nor control rats.

Changes in myocardial lipids by diabetes:

Tab.3 shows the distribution of fatty acid acyl residues in myocardial lipids of control, acutely and chronically diabetic rat hearts. As can be seen, the free fatty acid fraction (non-esterified fatty acids= NEFA) represented only a minor portion (less than 1%) of the total fatty acids. The amount of free fatty acids was increased by 25 and 53% in the acutely and chronically diabetic rat heart, respectively. From the fatty acid composition (Tab.4) it can be seen that in control hearts palmitic and stearic acid represent about 50%, oleic and linoleic acid about 13 and 9% of the total myocardial free fatty acids in agreement with data given by others (20,28,29). The increase in free fatty acids in diabetes was mainly caused by accumulation

experimental group	6-keto-PGF_{1a} (nmol/l)	change (%)
control	0.51 ± 0.10	-
acutely diabetic	0.27 ± 0.11	- 47
chronically diab.	0.26 ± 0.09	- 49

Tab.1: Concentration of 6-keto-$PGF_{1\alpha}$ (nmol/l) in the cardiac effluates of control, acutely and chronically diabetic rats. Hearts were isolated and perfused at constant pressure (60 mm Hg) with Krebs-Henseleit buffer. The cardiac effluate was collected between the 30 and 45 min. of perfusion time. 6-keto-$PGF_{1\alpha}$ was determined by RIA (n=5).

ARACHIDONIC ACID INDUCED PROSTAGLANDIN RELEASE (45 µg)					
Parameter	GROUP				
	Control n=5 (ng)	Acutely diabetic n=7 (ng)	Acutely diab. / Control	Chronically diabetic n=5 (ng)	Chronically diab. / Control
PGI_2	130 ± 19	297 ± 50	2.3	348 ± 88	2.7
PGE_2	15 ± 5	36 ± 8	2.4	91 ± 12	6.1
$\frac{PGI_2}{PGE_2}$	8.7	8.2	0.9	3.8	0.4

Tab.2: Release of prostacyclin and PGE_2 from control, acutely and chronically diabetic rat hearts after application of arachidonic acid (45 µg).

Prostacyclin and PGE_2 are expressed in ng, mean ± SEM. Experimental details are given in legend to fig.1. and in the Method section.

of palmitic, oleic and linoleic acid paralleled by a reduction of the amount of arachidonic acid in the acutely diabetic, but not in the chronically diabetic rat heart. Accordingly, the percentage of most fatty acids was reduced with the exception of oleic and linoleic acid in the acutely diabetic heart and of linoleic and pentadecanoic acid in the chronically diabetic state. Whereas the percentage of arachidonic acid was diminished for about 40% in the acutely diabetic heart, control and chronically diabetic hearts did not differ in the portion of arachidonic acid.

The amount of triglycerides calculated from the fatty acid acyl residues measured by gas chromatography or enzymatically determined by the glycerolkinase method after TLC and alkaline saponification agreed well (not shown). Both methods indicated a 3-fold increase in triglycerides in the acutely SZ-diabetic heart as compared to controls, but not in the chronically diabetic state (Tab.3.) Thus, triglycerides contained in acutely diabetic hearts about 11 %, in chronically diabetic hearts, however, 30 % of the total fatty acid acyl residues found. Additional bands on TLC plates could be identified as mono- and diglycerides by comparison with external standards (1,2- and 1,3-diglycerides, monoglycerides), but represented only minor fractions.

The absolute and relative composition of triglycerides is shown in Tab.5. Palmitic and stearic acid represented about 42% of the fatty acid acyl residues in triglycerides. The portion (%) of oleic and linoleic acid in triglycerides was nearly doubled as compared to the free fatty acid fraction. The amounts of all fatty acids in triglycerides were increased by the induction of the acutely diabetic state, most prominently of palmitic, stearic, oleic and linoleic acid. The absolute amount of arachidonic acid was slightly elevated.

The relative composition of the fatty acid spectrum showed an increase in oleic and linoleic acid, but a reduction of the percentage of all other fatty acids. In chronically diabetic hearts

Fatty acid acyl residues in :	control (nmol/g w.w.)	acutely diabetic (nmol/g w.w.)	chronically diabetic (nmol/g w.w.)
free fatty acids	183 ± 11	230 ± 15 +	281 ± 37 +
triglycerides	5341 ± 1177	17373 ± 551 +	5479 ± 1109 +
phospholipids	41287 ± 1226	41303 ± 1343	43837 ± 1206

Tab.3: Fatty acid distribution between myocardial free fatty acids, triglycerides and phospholipids in control and diabetic rat hearts.

The lipids were extracted by chloroform/methanol and separated by TLC. After esterification the fatty acids were determined by the gas chromatographic method described. Data are expressed as mean ± SEM of 6 experiments. + $p < 0.05$ vs. control

Fatty acids	control	acutely diabetic		chronically diabetic	
	% of sum	% of sum	% change	% of sum	% change
C 14	6.3 ± 0.5	4.3 ± 0.4 +	- 31 +	4.8 ± 0.4 +	- 24 +
C 15	4.8 ± 0.3	2.5 ± 0.2 +	- 48 +	10.9 ± 2.2 +,++	+ 126 +
C 16	36.4 ± 1.8	37.2 ± 0.8	2	32.3 ± 2.2	- 11
C 16:1	5.9 ± 0.7	4.0 ± 1.6	- 32	1.8 ± 0.3 +	- 70 +
C 18	20.0 ± 2.7	16.3 ± 0.9	- 18	18.3 ± 1.2	- 9
C 18:1	13.0 ± 1.4	16.5 ± 0.7	+ 27	11.4 ± 0.4 ++	- 13
C 18:2	9.4 ± 2.8	18.3 ± 0.8 +	+ 95 +	14.9 ± 1.3 +	+ 59 +
C 18:3	1.6 ± 0.4	0.8 ± 0.1 +	- 48 +	1.3 ± 0.3	- 19
C 20:3	traces	traces		traces	
C 20:4	4.3 ± 0.7	2.5 ± 0.4 +	- 42 +	4.5 ± 1.0	+ 5

Tab.4: Fatty acid composition of free fatty acids in control and diabetic hearts.

Free fatty acids were isolated and determined as described in the Method section. The data given as mean ± SEM of 5-6 experiments. The percentages were calculated from the amount of the individual fatty acids and the sum of fatty acids taken as 100%. + $p < 0.05$ vs. control

the amounts of the fatty acid acyl residues as well as the fatty acid spectrum resembled that of controls. The portion of linoleic and pentadecanoic acid was, however, elevated and the percentage of palmitic and palmitoleic acid reduced as compared to the control.
The absolute amount of fatty acid acyl residues in the phospholipid fraction (Tab.3 and 5) was not significantly changed by the diabetic state. Thus, the phospholipid fraction contained in controls and chronically diabetic hearts about 90%, in the acutely diabetic state about 70% of the total amount of fatty acid acyl residues found. The diabetic state only slightly influenced the amount of fatty acid acyl residues in the phospholipid fraction and the composition of the fatty acid spectrum. The arachidonic acid content of myocardial phospholipids was not reduced by the diabetic state.

DISCUSSION

Our results show that the basal release of prostacyclin is reduced in the diabetic heart and, thus, confirm data presented by others demonstrating a reduced prostacyclin synthesis by vessels of diabetic patients and experimentally diabetic rats 10-13,16, 17). The significance of this reduction in basal prostacyclin release is, however, not yet clear, since the actual release of PGs is strongly influenced by the experimental conditions (lesions and thrombin release by the preparation of vessel wall segments, heparinization (31), equilibration time and conditions after preparation). Therefore, Granström recently pointed out (32) that the estimation of PG-synthetic capacity of a certain tissue may be a more reliable approach to the study of tissue PGs than the determination of actual in situ PG level, an approach, which demands higher, but not arrythmogenic dosages of arachidonic acid to reach concentrations of arachidonic acid saturating for its different biological pathways (for review 33).

Fatty acids	control	acutely diabetic		chronically diabetic	
	% of sum	% of sum	% change	% of sum	% change
C 14	2.3 ± 0.2	1.2 ± 0.1 +	- 48 +	1.8 ± 0.3	- 24
C 15	0.9 ± 0.2	0.4 ± 0.1 +	- 60 +	1.7 ± 0.6 ++	+ 93 ++
C 16	34.1 ± 0.9	30.3 ± 0.8 +	- 11 +	29.8 ± 0.9 +	- 13 +
C 16:1	4.4 ± 0.6	2.6 ± 0.4 +	- 41 +	2.2 ± 0.6 +	- 50 +
C 18	8.5 ± 0.8	6.9 ± 0.4	- 19	8.4 ± 0.9	- 1
C 18:1	20.7 ± 0.6	26.0 ± 0.3 +	+ 26 +	21.9 ± 0.9 ++	+ 6 ++
C 18:2	25.4 ± 1.6	30.6 ± 1.0 +	+ 20 +	30.9 ± 1.0 +	+ 21 +
C 18:3	0.9 ± 0.2	0.7 ± 0.1	- 22	1.3 ± 0.1 ++	+ 36 ++
C 20:3	traces	traces		traces	
C 20:4	2.8 ± 0.6	1.2 ± 0.2 +	- 57 +	2.1 ± 0.4	- 26

Tab.5: Fatty acid composition of triglycerides in control and diabetic hearts.

Experimental details are given in the legend to Tab.2.

Fatty acids	control	acutely diabetic		chronically diabetic	
	% of sum	% of sum	% change	% of sum	% change
C 14	0.2 ± 0.02	0.2 ± 0.1	31	0.1 ± 0.01	-29
C 15	1.2 ± 0.1	1.8 ± 0.1 +	54 +	0.2 ± 0.01 +,++	-87 +
C 16	13.8 ± 0.4	12.1 ± 0.2 +	-13 +	13.2 ± 0.5 ++	- 5
C 16:1	0.3 ± 0.04	0.3 ± 0.1	-	0.2 ± 0.02 ++	-34
C 18	24.3 ± 0.3	25.1 ± 0.4	3	26.2 ± 0.5 +	8 +
C 18:1	6.0 ± 0.5	6.2 ± 0.3	3	6.2 ± 0.3	3
C 18:2	33.2 ± 0.7	34.7 ± 1.6	4	33.1 ± 0.3	0.3
C 18:3	0.2 ± 0.03	0.2 ± 0.02	-	0.2 ± 0.02	-
C 20:3	0.6 ± 0.1	0.6 ± 0.04	-	0.6 ± 0.1	-
C 20:4	20.8 ± 0.4	20.1 ± 1.1	-3	20.0 ± 0.5	- 4

ab.6: Fatty acid composition of phospholipids in control
ad diabetic hearts.

xperimetal details are given in legend to Tab.2.

In fact, in contrast to the actual prostacyclin release, the PG-biosynthetic capacity was not impaired in the diabetic heart, neither under acute and ketoacidotic nor under chronically diabetic conditions. In both series the formation of prostacyclin and PGE_2 was dose-dependent on the availability of exogenous arachidonic acid. If the same dosages were applied, diabetic hearts released even more PGs than controls. Comparing the acutely-ketoacidotic with a more chronically diabetic state, the rate of prostacyclin formation was not significantly different in both states, however, the synthesis of PGE_2 was further increased in the chronically diabetic state. Thus, an irreversible inhibition of myocardial cyclooxygenase by lipidperoxides as discussed by (34) can be excluded from our data, at least for the two diabetic states used in this study. It follows that in the diabetic heart the enzymatic capacity of the cyclooxygenase reaction is not impaired by diabetes, but that the availability of free arachidonic acid, which had to be liberated from complex lipids (33), limits PG-synthesis in diabetes.

Since not only an absolute loss of arachidonic acid in the phospholipid fraction, but also a general shift in the fatty acid composition might contribute to changes in the availability of free arachidonic acid, the diabetes induced changes in the fatty acid composition of myocardial lipids has been studied.

In controls, the amount of myocardial free fatty acids was very low in agreement with published data (18,20,28,29), but was elevated by both diabetic states. In hearts from alloxan-diabetic dogs, Liu and Spitzer (20) observed a similar increase of the free fatty acids. The significance of this increase in myocardial free fatty acids is not yet clear. An unfavourable influence of free fatty acids on adenine-nucleotide translocase (35), oxidative phosphorylation uncoupling (36) and on calcium depending processes (38) has been discussed. It is, however, questionable whether the amount of free fatty acids measured in control and diabetic hearts is sufficient to produce these effects, since it has to expected that most of the free fatty

acids are bound to cellular protein (38). We do not think that the described changes in free fatty acids observed in diabetic hearts result in alterations of the concentration of non-protein bound free fatty acids sufficient to evoke the deleterious effects of free fatty acids mentioned.
Whereas mono- and diglycerides represented minor fractions of total glycerides, triglycerides were more than doubled in acutely diabetic hearts in agreement with results of others (18,19). This accumulation of triglycerides cannot be explained by hyperlipidaemia associated with the diabetic state (39,40) alone, since in chronically diabetic hearts an accumulation of triglycerides has not been observed in spite of the presence of hyperlipidaemia. A ketotic-diabetic state leading to an inhibition of lipolysis and ß-oxidation of long chain fatty acids seems to be a precondition for an accumulation of triglycerides in the diabetic heart, in agreement with other experimental data (21,40).
The total amount of free fatty acid acyl residues measured in the total phopholipid fraction agreed well with data given in the literature (18), but was not influenced by either the acutely or chronically diabetic state in contrats to the free fatty acid and triglyceride fractions. Therefore, and since phosphaditylcholine and -ethanolamine represent about 30% of total phospholipids and are the main source for arachidonic acid (33), we did not differentiate between the various phospholipids.

Summarizing the influence of diabetes on the fatty acid composition of the lipid fractions studied, changes in the fatty acid composition were only observed in the free fatty acid and triglyceride fractions, but not in the phospholipids. Since the mass of neutral lipids and free fatty acids does not match that of phospholipids and the fatty acid compostion of phospholipids was not influenced by diabetes, we think that the diminuition of the arachidonic acid content in triglycerides and free fatty

acids as well as the increase in oleic and linoleic acid reflects more changes in the turnover of these fractions, but did not interfere with the availability of arachidonic acid for PG synthesis.

Therefore, we believe that the described reduced PG-synthesis by diabetes cannot be explained by changes in the arachidonic acid content, the immediate precursor of PG formation, nor by substrate competition as discussed by Ten Hoor et al. (41), which should be associated with a shift in the PG release induced by arachidonic acid in the diabetic heart not observed by us. Furthermore, our data clearly exclude a disturbancy in the direct metabolic pathways leading to prostacyclin and PGE_2 release. The regulation of phospholipase activity which mainly controls the amount of arachidonic acid for PG formation (34,42) seems, therefore, to represent the key for the understanding of diabetic induced changes in PG synthesis. The increased cardiac capacity of the diabetic heart for formation of prostaglandins reported here might represent a compensatory mechanism that tries to antagonize the increased thromboxane A_2 release seen in the diabetic state.

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